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In re

Patent Application of

James W. Schumm, et. al.

Serial No.: Unknown

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I, Diane J. Frauchiger, hereby certify that this correspondence is being deposited with the US Postal Service as Express Mail Post Office to Addressee Serial No. EL453985016US addressed to Assistant Commissioner for Patents, BOX PATENT APPLICATION, Washington, D.C. 20231, on the date of my signature.

Diane J. Frauchiger
Signature

April 21, 2001
Date of Signature

"MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI"

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
BOX PATENT APPLICATION
Washington, D.C. 20231

Sir:

This application is a continuation under 37 CFR 1.53(b) of U.S. Patent Application Serial No. 09/327,229, filed June 7, 1999, to be issued on April 24, 2001 as U.S. Patent No. 6,221,598, a continuation of U.S. Patent Application Serial No. 08/316,549, filed September 30, 1994, now abandoned. Prior to examination on the merits, and calculation of filing fees due with the above-identified application, please amend the subject application as follows:

In the Specification

Please amend the specification as follows:

On page 1, immediately before "FIELD OF THE INVENTION", please add the following new paragraph and heading:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. Patent Application Serial No. 09/327,229, filed June 7, 1999 now U.S. patent 6,221,598, which is a continuation application of U.S. Patent Application Serial No. 08/316,544, filed September 30, 1994, now abandoned.

Replace the second full paragraph on page 3 with the following:

Ballabio et al. (1991), disclose a single-tube, multiplex allele-specific PCR test using two different dye-tagged fluorescent primers for detection of the ▲F508 cystic fibrosis mutation.

On page 7, after line 18 and before line 19, add the following new paragraph:

Fig. 24 is a photograph showing the silver stained detection of the multiplex amplification in example 24.

Replace the first full paragraph on page 11 with the following:

The primers must also be designed so that the size of the resulting amplification products differ in length, thereby facilitating assignment of alleles to individual loci during detection. Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. The synthesis of the primers is conducted by procedures known to those skilled in the art.

Replace the third full paragraph on page 18 with the following:

In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMVWFA31 simultaneously in a single reaction vessel. The PCR amplifications were performed in 25µl volumes using 25ng template, 0.04U *Taq* DNA Polymerase/µl, 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM $MgCl_2$ and 200µM each of dATP, dCTP, dGTP and dTTP), and using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 1, as described in Example 1, was employed. Eight amplification primers were used in combination, including 1µM each HUMCSF1PO primer 2 [SEQ. ID._6] and fluorescein-labeled

primer 1 [SEQ. ID. 5], 0.15μM each HUMTPOX primer 1 [SEQ. ID. 29] and fluorescein-labeled primer 2 [SEQ. ID. 30], 0.2μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27], and 1μM each HUMVWFA31 primer 1 [SEQ. ID. 31] and fluorescein-labeled primer 2 [SEQ. ID. 32].

<u>In the Claims</u>

Please cancel without prejudice claims 1 through 20.

Please add the following new claims 21-55:

21. (New) A method of simultaneously determining the alleles present in at least two short tandem repeat loci from one or more DNA samples, comprising:

a. obtaining at least one DNA sample to be analyzed;

b. selecting a set of at least two short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the at least two short tandem repeat loci in the set comprises at least two loci selected from the group consisting of:

HUMTH01 and HUMCSF1PO;
HUMTH01 and HUMCD4;
HUMTH01 and HUMTPOX;
HUMFBA01 and HUMFABP;
HUMF13A01 and HUMMYOPK (Myotonic);
HUMFBA01 and HUMBFXIII (F13B);
HUMBFXIII (F13B) and HUMFESFPS;
HUMBFXIII (Fl3B) and HUMLIPOL;
HUMHPRTB and HUMFESFPS;
HUMHPRTB and HUMBFXIII (F13B);
HSAC04 (ACTBP2) and HUMCYP19;
HSAC04 (ACTBP2) and HUMFABP;
HUMCYP19 and HUMPLA2Al; and
HUMCSF1PO and HUMTPOX;

c. co-amplifying the set of at least two short tandem repeat loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

d. evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

22. (New) The method of claim 21 wherein the set of at least two short tandem repeat loci co-amplified therein is a set of at least three loci selected from the group consisting of:

HUMTPOX, HUMVWFA31 and HUMCSF1PO;

HUMHPRTB, HUMFESFPS and HUMVWFA31;

HSAC04 (ACTBP2), HUMCYP19 and HUMPLA2Al;

HUMAPOA2, HUMCYP19 and HUMPLA2Al;

HUMCD4, HUMCSF1PO and HUMTH01;

HUMCYP19, HUMFABP and HUMPLA2Al;

HUMCYP19, HUMHPRTB and HUMPLA2Al;

HUMHPRTB, HUMFESFPS and HUMLIPOL;

HLTMF13A01, HUMFABP and HUMCD4;

HUMHPRTB, HUMBFXIII (Fl3B) and HUMPLA2A1;

HUMHPRTB, HUMBFXIII (F13B) and HUMTPOX;

HUMHPRTB, HUMBFXIII (F13B) and HUMFESFPS;

HUMBFXIII (F13B), HUMFESFPS and HUMLIPOL;

HUMCSF1PO, HUMTPOX and HUMCD4;

HUMHPRTB, HUMFESFPS and HUMMYOPK (Myotonic);

HUMCSF1PO, HUMTH01 and HUMCD4;

HUMCSF1PO, HUMTH01 and HUMVWFA31; and

HUMHPRTB, HUMBFXIII (Fl3B) and HUMLIPOL.

23. (New) The method of claim 21 wherein the set of at least two short tandem repeat loci co-amplified therein is a set of at least three loci comprising: HUMTPOX, HUMTH01 and HUMCSF1PO;

24. (New) The method of claim 21 wherein the set of at least two short tandem repeat loci co-amplified therein is a set of at least four loci selected from the group consisting of:

HUMHPRTB, HUMFESFPS, HUMBFXIII (Fl3B) and HUMLIPOL; and

HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4.

25. (New) The method of claim 21 wherein the set of at least two short tandem repeat loci co-amplified therein is a set of at least four loci comprising: HUMCSF1PO, HUMTPOX, HUMTH01 and HUMVWFA31.

26. (New) The method of claim 21 wherein in step (b), the at least two loci are co-amplified by multiplex polymerase chain reaction.

27. (New) The method of claim 21 wherein the at least two loci are co-amplified using at least one oligonucleotide primer pair wherein at least one of each of the pair of primers used in the multiplex amplification reaction has a sequence selected from one of the groups of sequences consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;

SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;

SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;

SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;

SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4

SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;

SEQ ID. NO.13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;

SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;

SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;

SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMHPRTB;

SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);

SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;

SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;

SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;

SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and

SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

28. (New) The method of claim 21, wherein the amplified alleles are evaluated in step (e) by separating the alleles and comparing the separated alleles to a size standard selected from a DNA size marker or a locus-specific allelic ladder.

29. (New) The method of claim 21, further comprising the step of separating the alleles by denaturing polyacrylamide gel electrophoresis.

30. (New) The method of claim 29 wherein the separated alleles are detected by silver staining.

31. (New) The method of claim 29 wherein the separated alleles are detected by fluorescence detection.

32. (New) The method of claim 21 further comprising a step of identifying primers for co-amplifying each locus in the set of loci selected in step (b) such that the amplified alleles produced in the multiplex reaction of step (c) do not overlap.

33. (New) The method of claim 21 wherein the at least one DNA sample to be analyzed is selected from the group consisting of blood, semen, vaginal cells, hair, saliva, urine or other tissue, placental cells or fetal cells present in amniotic fluid and mixtures of body fluids.

34. (New) A kit for simultaneously analyzing short tandem repeat sequences in at least two loci, comprising:

a container containing oligonucleotide primers for each locus in a set of at least two short tandem repeat loci, wherein the at least two short tandem repeat loci in the set comprises at least two loci selected from the group consisting of:

HUMTH01 and HUMCSF1PO;
HUMTH01 and HUMCD4;
HUMTH01 and HUMTPOX;
HUMF13A01 and HUMFABP;
HUMF13A01 and HUMMYOPK (Myotonic);
HUMF13A01 and HUMBFXIII (F13B);
HUMBFXIII (F13B) and HUMFESFPS;
HUMBFXIII (F13B) and HUMLIPOL;
HUMHPRTB and HUMFESFPS;
HUMHPRTB and HUMBFXIII (F13B);
HSAC04 (ACTBP2) and HUMCYP19;
HSAC04 (ACTBP2) and HUMFABP;

HUMCYP19 and HUMPLA2A1; and
HUMCSF1PO and HUMTPOX.

35. (New) The kit of claim 34, wherein the container contains oligonucleotide primers designed to co-amplify a set of at least three short tandem repeat loci, wherein the set of at least four short tandem repeat loci comprises at least three loci selected from the group consisting of: HUMTPOX, HUMTH01 and HUMCD4;
HUMTPOX, HUMTH01 and HUMVWFA31;
HUMTPOX, HUMVWFA31 and HUMCSF1PO;
HUMHPRTB, HUMFESFPS and HUMVWFA31;
HSAC04 (ACTBP2), HUMCYP19 and HUMPLA2A1;
HUMAPOA2, HUMCYP19 and HUMPLA2A1;
HUMCD4, HUMCSF1PO and HUMTH01;
HUMCYP19, HUMFABP and HUMPLA2Al;
HUMCYP19, HUMHPRTB and HUMPLA2A1;
HUMHPRTB, HUMFESFPS and HUMLIPOL;
HUMF13AO1, HUMFABP and HUMCD4;
HUMHPRTB, HUMBFXIII (Fl3B) and HUMPLA2Al;
HUMHPRTB, HUMBFXIII (F13B) and HUMTPOX;
HUMHPRTB, HUMBFXIII (Fl3B) and HUMFESFPS;
HUMBFXIII (F13B), HUMFESFPS and HUMLIPOL;
HUMCSF1PO, HUMTPOX and HUMCD4;
HUMHPRTB, HUMFESFPS and HUMMYOPK (Myotonic);
HUMCSF1PO, HUMTH01 and HUMCD4;
HUMCSF1PO, HUMTH01 and HUMVWFA31; and
HUMHPRTB, HUMBFXIII (Fl3B) and HUMLIPOL.

36. (New) The kit of claim 34, wherein the kit contains oligonucleotide primers designed to co-amplify a set of at least three short tandem repeat loci, comprising: HUMCSF1PO, HUMTPOX, and HUMTH01.

37. (New) The kit of claim 36, wherein the kit contains oligonucleotide primers designed to co-amplify the set of at least three short tandem repeat loci, further comprising HUMVWFA31.

38. (New) The kit of claim 34, wherein the kit contains oligonucleotide primers designed to co-amplify a set of at least four short tandem repeat loci, wherein the set of at least four short tandem repeat loci comprises at least two loci selected from the group consisting of:

HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4; and

HUMHPRTB, HUMFESFPS, HUMBFXIII (F13B), and HUMLIPOL.

39. (New) The kit of claim 34 wherein each of the oligonucleotide primers in the kit is designed to hybridize with an allele of one of the loci in the set of at least two short tandem repeat loci, wherein the sequence of at least one primer is selected from the group consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;

SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;

SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;

SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;

SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4

SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;

SEQ ID. NO.13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;

SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;

SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;

SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMHPRTB;

SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);

SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;

SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;

SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;

SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and

SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

40. (New) A method of simultaneously determining the alleles present in a set of short tandem repeat loci from one or more DNA samples, comprising:

a. obtaining at least one DNA sample to be analyzed;

b. selecting a set of short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, comprising HUMCSF1PO, HUMTPOX, and HUMTH01;

c. co-amplifying the set of short tandem repeat loci in a multiplex amplification reaction, using wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

d. evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

41. (New) The method of claim 40, wherein the multiplex reaction is carried out using oligonucleotide primer pairs with primer pair sequences comprising: SEQ ID. NO. 5 and SEQ ID. NO. 6; SEQ ID. NO. 29 and SEQ ID. NO. 30; and SEQ ID. NO. 27 and SEQ ID. NO. 28.

42. (New) The method of claim 40, wherein the oligonucleotide primer pairs having the sequences SEQ ID. NO. 5 and SEQ ID. NO. 6, and SEQ ID. NO. 29 and SEQ ID. NO. 30 are present in a concentration of about 0.2 μM, and the oligonucleotide primer pairs SEQ ID. NO. 27 and SEQ ID. NO. 28 are present in a concentration of about 0.6 μM.

43. (New) The method of claim 40, wherein the set of loci co-amplified further comprises HUMVWFA31.

44. (New) The method of claim 40, wherein the multiplex reaction is carried out using oligonucleotide primer pairs with primer pair sequences comprising: SEQ ID. NO. 5 and SEQ ID. NO. 6, SEQ ID. NO. 29 and SEQ ID. NO. 30, SEQ ID. NO. 27 and SEQ ID. NO. 28, and SEQ ID. NO. 31 and SEQ ID. NO. 32.

45. (New) The method of claim 44, wherein the oligonucleotide primer pairs SEQ ID. NO. 5 and SEQ ID. NO. 6 are present in a concentration of about 1 μM; oligonucleotide primer pairs SEQ ID. NO. 29 and SEQ ID. NO. 30 are present in a concentration of about 0.15 μM, oligonucleotide primer pairs SEQ ID. NO. 27 and SEQ ID. NO. 28 are present in a concentration of about 0.2 μM, and oligonucleotide primer pair SEQ ID. NO. 31 and SEQ ID. NO. 32 are present in a concentration of about 1μM.

46. (New) The method of claim 40, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by silver staining.

47. (New) The method of claim 40, wherein the multiplex amplification reaction includes oligonucleotide primers for each locus in the set of loci selected in step (b), wherein at least one of the oligonucleotide primers for each locus is fluorescently labeled.

48. (New) A method of simultaneously determining the alleles present in a set of at least three loci from one or more DNA samples, comprising:

a. obtaining at least one DNA sample to be analyzed;

b. selecting a set of at least three short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the at least three short tandem repeat loci in the set comprises at least two loci selected from the group consisting of:

HUMTH01 and HUMCSF1PO;

HUMTH01 and HUMCD4;

HUMTH01 and HUMTPOX;

HUMF13A01 and HUMFABP;

HUMFBA01 and HUMMYOPK (Myotonic);

HUMFBA01 and HUMBFXIII (F13B);

HUMBFXIII (F13B) and HUMFESFPS;

HUMBFXIII (F13B) and HUMLIPOL;

HUMHPRTB and HUMFESFPS;

HUMHPRTB and HUMBFXIII (Fl3B);

HSAC04 (ACTBP2) and HUMCYP19;

HSAC04 (ACTBP2) and HUMFABP;

HUMCYP19 and HUMPLA2Al; and

HUMCSF1PO and HUMTPOX;

c. co-amplifying the at least three loci in the DNA sample in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

d. evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

49. (New) The method of claim 48, wherein the amplified alleles produced in step (c) do not physically overlap when separated by electrophoresis.

50. (New) The method of claim 48, wherein the set of at least three loci is co-amplified in step (c) using at least one oligonucleotide primer selected from the group consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;

SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;

SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;

SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;

SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4

SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;

SEQ ID. NO.13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;

SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;

SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;

SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMHPRTB;

SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);

SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;

SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;

SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;

SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and

SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

51. (New) A method of simultaneously determining the alleles present in at least two short tandem repeat loci from one or more DNA samples, comprising:

a. obtaining at least one DNA sample to be analyzed;

b. selecting a set of at least two short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the at least two short tandem repeat loci in the set comprises at least two loci selected from the group consisting of:

HUMTH01 and HUMCSF1PO;

HUMTH01 and HUMCD4;

HUMTH01 and HUMTPOX;

HUMFBA01 and HUMFABP;

HUMF13A01 and HUMMYOPK (Myotonic);

HUMF13A01 and HUMBFXIII (F13B);

HUMBFXIII (F13B) and HUMFESFPS;

HUMBFXIII (Fl3B) and HUMLIPOL;

HUMHPRTB and HUMFESFPS;

HUMHPRTB and HUMBFXIII (F13B);

HSAC04 (ACTBP2) and HUMCYP19;

HSAC04 (ACTBP2) and HUMFABP;

HUMCYP19 and HUMPLA2A1; and

HUMCSF1PO and HUMTPOX;

c. providing at least one pair of primers for each locus in the set of at least two short tandem repeat loci, wherein at least one of each of the pair of primers has a sequence selected from one of the groups of sequences consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;

SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;

SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;

SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;

SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4

SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;

SEQ ID. NO.13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;

SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;

SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;

SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMHPRTB;

SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);

SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;

SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;

SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;

SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and

SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

d. co-amplifying the set of at least two short tandem repeat loci in a multiplex amplification reaction using the at least one primer for each locus, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

e. evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

52. (New) The method of claim 51, wherein the set of loci selected in step (a) and the at least one primer provided in step (b) are designed such that the amplified alleles produced in the multiplex reaction of step (c) do not overlap when the amplified alleles are separated by electrophoresis.

53. (New) The method of claim 51, wherein the amplified alleles are detected by silver staining.

54. (New) The method of claim 51, wherein a least one primer for each locus used in the multiplex amplification reaction is fluorescently-labeled, and the amplified alleles are evaluated in step (e) by fluorescent detection.

55. (New) A kit for simultaneously analyzing short tandem repeat sequences in at least two loci, comprising:

a container containing oligonucleotide primers for each locus in a set of at least two short tandem repeat loci, wherein the at least two short tandem repeat loci in the set comprises loci selected from the group consisting of:

HUMTH01 and HUMCSF1PO;

HUMTH01 and HUMCD4;

HUMTH01 and HUMTPOX;

HUMF13A01 and HUMFABP;

HUMF13A01 and HUMMYOPK (Myotonic);

HUMF13A01 and HUMBFXIII (F13B);

HUMBFXIII (F13B) and HUMFESFPS;

HUMBFXIII (F13B) and HUMLIPOL;

HUMHPRTB and HUMFESFPS;

HUMHPRTB and HUMBFXIII (F13B);

HSAC04 (ACTBP2) and HUMCYP19;

HSAC04 (ACTBP2) and HUMFABP;

HUMCYP19 and HUMPLA2A1; and

HUMCSF1PO and HUMTPOX;

wherein each of the oligonucleotide primers in the kit is designed to hybridize with an allele of one of the loci in the set of at least two short tandem repeat loci, wherein the sequence of each primer is selected from the group consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;

SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;

SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;

SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;

SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4;

SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;

SEQ ID. NO.13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;

SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;

SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;

SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMHPRTB;

SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);

SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;

SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;

SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;

SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and

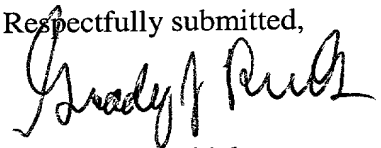
SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

Remarks

Applicants respectfully submit that the claim amendments, above, are identical to claim amendments made to U.S. patent application no. 08/316,544 at the time the parent of the present application (i.e., U.S. application no. 09/327,229) was filed.

Applicants submit, furthermore, that all amendments to the specification, except for the addition of the "Cross Reference to Related Applications" paragraph, were also made at the time of filing the parent application. Applicants submit that none of the claim amendments adds any new matter to the original application, as filed.

Respectfully submitted,



Grady J. Frenchick
Reg. No. 29,018

Date: April 20, 2001

Docket No.: 016026-9238
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Version with Markings to Show Changes Made

The amended paragraphs indicate deletions by ~~strikeout~~ and insertions by underlining.

In the specification, page 3, second full paragraph.

> *Ballabio et al.* (1991), disclose a ~~single-tube multiplex allele-specific~~ single-tube, multiplex allele-specific PCR test using two different dye-tagged fluorescent primers for detection of the ▲F508 cystic fibrosis mutation.

In the specification, page 11, first full paragraph.

> The primers must also be designed so that the size of the resulting amplification products differ in length, thereby facilitating assignment of alleles to individual loci during detection. Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or ~~requirement~~ the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. The synthesis of the primers is conducted by procedures known to those skilled in the art.

In the specification, page 18, third full paragraph.

> In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMVWFA31 simultaneously in a single reaction vessel. The PCR amplifications were performed in 25µl volumes using 25ng template, 0.04U *Taq* DNA Polymerase/µl, 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM $MgCl_2$ and 200µM each of dATP, dCTP, dGTP and dTTP), and using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 1, as described in Example 1, was employed. Eight

amplification primers were used in combination, including 1μM each HUMCSF1PO primer 2 ~~[SEQ. ID. 5]~~ [SEQ. ID. 6] and fluorescein-labeled primer 1 [SEQ. ID. 5], 0.15μM each HUMTPOX primer 1 [SEQ. ID. 29] and fluorescein-labeled primer 2 [SEQ. ID. 30], 0.2μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27], and 1μM each HUMVWFA31 primer 1 [SEQ. ID. 31] and fluorescein-labeled primer 2 [SEQ. ID. 32].